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The role of membranes and phase boundaries in generating electric currents in living tissues.

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UNIVERSITY OF LOUISVILLE

THE ROLE OF MEMBRANES AND PHASE BOUNDARIES IN
GENERATING ELECTRIC CURRENTS IN LIVING
TISSUES.

A Dissertation

Submitted to the Faculty

Of the Graduate School of the University of Louisville

In Partial Fulfillment of the

Requirements for the Degree

Of Master of Science

Department of Physiology and Pharmacology
(Biophysics)

By

Norman E. Epstein

1934

11 July '34 E.D.

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Norman E. Epstein.

GENERAL REMARKS

"I can see no reason for the pessimistic assumption that the artificial transformation of dead into living substance might not be accomplished sometime." * It is this spectacular but not unreasonable hope which has stimulated a widespread interest among the scientific investigators and speculators in biophysical phenomena.

While many of the processes which occur in living substances cannot be reconstructed for demonstration, still artificial models may be devised which do imitate and correspond to the physicochemical reactions taking place in the living system. It is true that the composition of these models may differ from that of the living; however, much can be learned from their behavior, and can serve as a guide to the ultimate discovery of the true nature of life.

At the present time most of the attention of biophysicists is centered on the behavior of membranes in generating electric currents in living tissues. It is the hope of the author that this paper will contribute to the advancement towards the understanding of this subject.

* J. Loeb, "Dynamics of Living Matter," Chicago, 1906.

HISTORICAL INTRODUCTION

Electrophysiology, as a science, had its beginning in the year 1786 in Galvani's famous discovery (1737-1798) which seemed largely to be accidental in nature. Galvani (2) was conducting some experiments on the muscles in the legs of frogs and his attention was called to the violent and spasmodic contractions of the muscles when they were touched in a certain way with a pair of prongs containing Cu and Zn. From this observation, Galvani assumed that the twitching of the muscle was due to some form of electricity. However, he was in error in believing that some inherent vital force created this electricity.

Count Allesandro Volta (1745-1827), professor of physics at Parvia, was able to explain almost as accurately as we know today, the real cause of the muscular contraction observed in Galvani's experiment. Volta, showed experimentally, that an electric current could be produced by placing a piece of copper and a piece of zinc in a solution of sulfuric acid or some other electrolyte. From this experiment Volta was able to conclude that in Galvani's work, the frog's leg played the role of an intermediate salt solution and did not create currents of its own account. Volta, however, was not entirely correct in his theory. He believed that the junction of the

two metals — Cu and Zn — in the cell arrangement was the only location where electricity was produced and also that living tissue never gives rise to electricity.

Fifty years later, after Volta's famous discovery, Du Bois Reymond (1818-1896), a frenchman, gave proof that organic tissues do produce electric currents. Du Bois Reymond (1), in attempting to explain the origin of these electric currents, adopted the theory of bipolar molecules which merely states that the smallest particles of living tissue are positively charged at one end and negatively at the other. Du Bois Reymond, in adopting this theory was greatly influenced by Weber's theory of "Molecular Magnets" and he tried to correlate his bipolar theory with that of Weber's which was the most discussed theory at that time. Reymond's theory is important merely because it had postulated that some substance present in tissue, a priori was the cause of the current detected.

Louis Hermann, a former pupil of Du Bois Reymond, condemned the conclusions of his former master. Hermann, in his "Alteration" theory, postulated that living tissue should be devoid of all electric potential differences and that potential differences should arise merely as a consequence of death, decay or degradation of life processes. To prove this basic idea that electricity was produced by degrading changes, Hermann showed that the injury current appeared within a definite

time after the injury was inflicted. He found this period of time to be extremely short, being about $1/1000$ of a second, and thus he interpreted it to be the time required for the cut surface of a muscle to pass from life to death.

Hermann's theory, however, contradicts simple physical evidence which shows that electrical potential differences exist at every boundary of differentiated living structures. His alteration theory could be true only if living tissue were homogeneous throughout.

In 1890 W. Ostwald, a well known physical chemist, introduced the hypothesis that membranes endowed with a differential permeability for positive and negative ions should be the peculiar cause of bioelectricity. At the present time, L. Michaelis is an ardent supporter of this hypothesis. His reasons for supporting it as well as many arguments against it will be discussed later, in much more detail.

Thus electrophysiologists have known for a long time that electric phenomena are associated with life and that they arise in living tissues. However many of the investigators consider bioelectricity as so closely associated with life processes that reproduction of anything remotely resembling these phenomena by synthetic means is considered impossible. This is certainly not true. It is quite possible to reproduce by artificial means many of the essential underlying conditions which are responsible for the generation of electric currents in

tissues, and to observe how such artificial systems produce potential differences in a similar manner. As a matter of fact no explanation of bioelectricity can be adequate unless it is based on an imitation of the natural phenomena. The ancient belief that all organic substances are produced by dint of the vital force has been gradually dying out since thousands of such substances are now being prepared synthetically.

Since it has been definitely proven that electric currents arise in living tissue, sensitive electrometers have been devised by means of which the electromotive force produced, when an electric current passes through a living tissue, has been accurately measured. Artificial models have been constructed containing systems similar to those found in living cells and their electromotive forces were measured. To the regret of the biophysicists, the electromotive forces produced in artificial systems were found to be ten times weaker than that of a living cell. In 1911 J. Loeb suggested to R. Beutner (3) that he investigate the potential difference between such substances as apples or leaves of the rubber plant instead of the potential difference of muscles or nerves. In the subsequent experiments R. Beutner made the important observation that the potential difference between the surface of an apple or a leaf was a maximum where the bounding liquid was pure water while the potential difference was depressed when a salt was added

to the water, the depressing effect on the potential difference increasing with the concentration of the salt.

A search was made for those substances in the cortex of an apple or leaf which might be responsible for these peculiar concentration effects on the potential difference. It was already known that the cuticula of the apple was a homogeneous wax-like covering consisting chemically of a mixture of fatty acids and higher alcohols and therefore lipoidal in character. R. Beutner, aiming at a further and more lucid explanation of the electromotive forces at plant cuticula found that there existed a potential difference at the boundary of lipoids which was similarly depressed and likewise, the more so, the higher the concentration of the salt.

The idea that lipoids are the substances responsible for the potential difference of tissues led R. Beutner to an extensive study of the potential difference at the boundary of water immiscible substances and various salt solutions. Cell systems composed of aqueous salt solutions and non-aqueous water immiscible conductors were set up. The aqueous salt solutions of varying concentrations used were sodium chloride, calcium chloride, potassium chloride, and sodium sulfate while phenol, salicylic aldehyde, cresol and guaiacol were used as central conductors. Results showed that the electromotive forces of these artificial cell systems were similar in strength

to those of living tissues. From these results, R. Beutner (1) concluded that it is only by interposing electrolytic conductors that are water immiscible between the aqueous solutions in the electrolytic cells that electromotive forces can be produced in a similar order of magnitude to those which appear in living tissues. To comprehend these phenomena a careful understanding of the phase boundary potential theory, which is discussed in detail in the next part of this paper, should be sought.

THE PHASE BOUNDARY POTENTIAL THEORY AND ITS APPLICATION.

The Phase Boundary Potential Theory and its Application.

The phase boundary potential theory is applicable to those systems mentioned above (see p. 7) which are set up by a non-aqueous substance between two solutions of the same salt having different concentrations. In these systems, the ratio of the ionic concentrations in the two neighboring phases determines the potential difference. We should expect therefore that whenever an effect of concentration appears, the concentration of the common ion in the second phase should be independent of the aqueous salt concentration or at least nearly independent. If these two ionic concentrations underwent parallel variations, as one might expect according to the law of partition, no electromotive effect should manifest itself. Experimental evidence shows however, that these two ionic concentrations do not always undergo parallel variation; hence the law of partition cannot be applied in its simplest form. This is clearly shown by the numerous experiments performed by R. Beutner (2).

Experimental Proof

Shaking experiments were performed according to the following procedure. In every series of experiments a constant volume of a particular water immiscible substance was shaken with an equal volume of salt solutions of different concentrations, all

other conditions remaining unchanged. The rise in conductivity was observed as a measure of ionic concentration which existed when the water immiscible substance was shaken with the aqueous salt solution. It appeared, very strikingly, in many cases that the conductivity of the central conductor was not greatly influenced when it was shaken with two salt solutions of highly different concentrations.

Example:

A solution of a salt of an organic base was shaken with salicylic aldehyde for several hours.

- (1) 12.5 c.c. of salicylic aldehyde
+ 50 c.c. 1/125 M dimethylaniline HCl
- (2) 12.5 c.c. of salicylic aldehyde
+ 50 c.c. 1/5 M dimethylaniline HCl

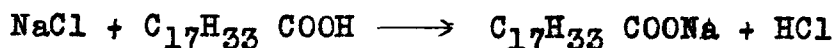
After several hours of shaking the conductivity of salicylic aldehyde, in both cases, was measured.

- | | |
|---|-------------------|
| (1) conductivity of salicylic aldehyde
before shaking | 0.7 rec. megohms |
| (2) conductivity of salicylic aldehyde
after shaking with 1/125 M of dimethylaniline HCl | 7.8 rec. megohms |
| (3) conductivity of salicylic aldehyde
after shaking with 1/5 M dimethylaniline HCl | 19.9 rec. megohms |

Whereas the proportion of the concentrations of the aqueous salt solutions was 1:25, the ratio of the conductivities of the two salicylic aldehydes was 1:2.5. From these proportions it

is apparent that the rise in conductivity of the salicylic aldehyde did not vary in the same ratio as the concentrations of the salt solutions and from this R. Beutner deduces that the law of partition does not function here in its simplest form.

If cresol plus oleic acid were used as a central conductor between various inorganic salt solutions we would also observe the effect of an electromotive force as soon as the concentration of the salts varied. According to the theory as stated above, the variability of the electromotive force of a system should depend on the constancy of the common ion in the water immiscible substance. If a system were set up where NaCl solutions of varying concentrations were used with cresol plus oleic acid, then a difference in potential would be produced due to the constancy of the Na^+ ions in the oleic acid. This constancy is effected in the following manner. The Na ion penetrates into the oleic acid and combines with it to form sodium oleate, a soap, the formation of this soap depending only on the amount of oleic acid present which almost always remains constant. This phase boundary phenomenon between oleic acid and the NaCl solution leads us to the assumption that a chemical reaction occurs between the water immiscible substance and the adjacent salt solution.



Such a chemical reaction seems contrary to a general chemical experience since HCl will decompose Na oleate to oleic

acid and NaCl. Nevertheless, the reaction proceeds in the manner indicated as can be demonstrated by titration experiments.

When a solution of oleic acid plus cresol is added to a sodium chloride solution and then the mixture is agitated, hydrochloric acid is formed and is detected by titration. Control experiments are necessary since cresol plus oleic acid gives off traces of an acid even to pure water when shaken with it, but when shaken with sodium chloride more acid is formed as demonstrated by the following tests.

20 c.c. of cresol plus 1% oleic acid
are shaken with
20 c.c. of water or 0.1 M NaCl solution
for four hours

After the shaking is completed the layers are separated by centrifuging and filtered. Equal quantities of the filtrates were then titrated, but before this was done as much sodium chloride was added to the water, which had been shaken with the cresol mixture, as was contained in the original sodium chloride-cresol mixture. In other words, the two solutions had the same NaCl content at the time they were titrated. Thus the possibility of a salt error in titration is excluded. The result was that the sodium chloride solution required five times as much more 0.01 M NaOH than the water solution.

In another experiment performed with cresol C.P. (this being less acidic) after two hours an additional 1.2 c.c. 0.01 M NaOH was required by the NaCl solution. After eight hours 2.25 c.c. 0.01 M NaOH more was required.

This considerable amount of excess acid formed in the sodium chloride solution shaken with the cresol plus fatty acid mixture can hardly be due to anything else but hydrochloric acid formation by interaction of sodium chloride and fatty acid.

We have demonstrated by titration the presence of HCl in the solution; the detection of the presence of soap (Na oleate) is all that is now needed to prove that this chemical reaction occurs. The question is, how can this be done? We can see whether the cresol mixture which has produced the acid contains soap by titrating it as an alkali. The cresol mixture after having been shaken with sodium chloride is now shaken with pure water. It should yield alkali, at least, more alkali than that cresol mixture which previously had been shaken with water only, and hence yielded less acid. This theoretical conclusion has also been verified as the following figures show.

After two hours 0.8 c.c. of 0.01 M sodium hydroxide (less than for the mixture shaken with sodium chloride) was required, while after eight hours 8.0 c.c. of 0.01 M sodium hydroxide (less than for the mixture shaken with sodium chloride) was required.

These experiments clearly show that sodium chloride can be split up into hydrochloric acid and a basic constituent.

Application of the Phase Boundary Potential Theory to Organic Bases Which Are Used as Central Conductors.

Thus far the phase boundary potential theory has been applied to non-aqueous organic fluids which contained a water insoluble acid or have had acid properties themselves.

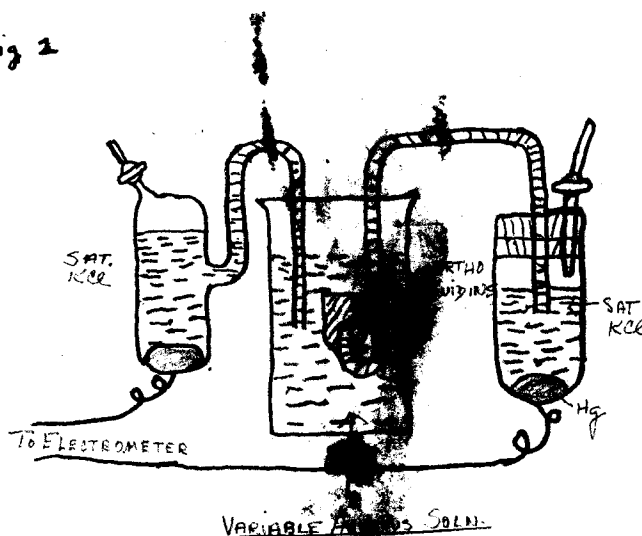
E.g.: salicylic aldehyde plus salicylic acid
 cresol plus oleic acid
 guaiacol plus oleic acid

If an organic base instead of an organic acid is used as a central conductor it should be expected to exhibit electromotive changes in the opposite direction. In fact, a certain base like ortho toluidine does exhibit an effect of concentration in the opposite direction. (Impurities may alter this change.)

Series of measurements were made with NaCl solutions. The experimental arrangement is shown in Fig. 1 . The ortho toluidine is poured into the hook-shaped electrode while NaCl solutions of varying concentrations are poured into the beakers and a measurement is made of the electromotive force of each solution. Connections to a measuring instrument (a sensitive electrostatic electrometer) are made by means of two calomel electrodes in the manner shown in the diagram. A variation of 0.079 volt was found for a change of concentration from 1/10

molecular to a 1/1250 molecular NaCl, the latter being on the positive side; hence the effect of concentration is opposite to that on salicylic aldehyde, with all neutral salts such an inverse electromotive effect can be observed particularly well with KSCN. The latter substance shows a change of 0.107 volts for a variation of the concentration from 1/10 molecular to 1/1250 molecular.

Fig 2



Shaking and Titration Experiments with Ortho Toluidine to Detect the Liberation of a Base.

According to the theory a chemical reaction should occur between the ortho toluidine and the various salt solutions whereby a base should be liberated. Shaking experiments were performed using equal quantities of ortho toluidine with the same quantities of various 0.1 M⁴ salt solution^s. These mixtures were shaken for several hours, then centrifuged for the separation of the layers and then filtered and titrated. Control experiments were used while salt errors were omitted by applying the same process which I have explained previously. Phenolphthalein was used as an indicator.

Observations

- I. With CaCl_2
 - 15 c.c. ortho toluidine + 15 c.c. M/10 CaCl_2
were shaken for several hours
 - On titration
 - 10 c.c. of the filtrate (CaCl_2) required 0.320 of
0.989 M/100 NaOH to turn phenolphthalein pink.
- II. 15 c.c. ortho toluidine + 15 c.c. of distilled water
were also shaken for several hours for control
purposes.
 - 10 c.c. of the filtrate (distilled water) was titrated
with 0.140 c.c. of 0.989 M/100 NaOH

From the experimental data one notices that the CaCl_2 solution after shaking was slightly acidic and required the addition of 0.320 of 0.989 M/100 NaOH to make it basic.

I. With LiCl the same phenomenon was observed.

15 c.c. ortho toluidine + 15 c.c. M/10 LiCl
were shaken for several hours

10 c.c. of filtrate required 0.270 c.c. of 0.989 M/100
NaOH for titration

II. 15 c.c. ortho toluidine + 15 c.c. of distilled water
shaken for several hours

10 c.c. of filtrate (distilled water) required 0.135 c.c.
of 0.989 M/100 NaOH for titration

When KI or KCNS were shaken with ortho toluidine, the liberation of a base was detected upon the addition of 3 drops of phenolphthalein to the filtrates.

Experimental Evidence

I. 15 c.c. ortho toluidine + 15 c.c. M/10 KCNS were shaken for several days. When to 10 c.c. of the filtered fluid 3 drops of phenolphthalein were added a deep permanent pink color was obtained, thus proving that a base was liberated during the shaking of ortho toluidine with KCNS. This agreed with the inverse electromotive effect of concentration which was most readily observed with KCNS and KI.

II. As a control experiment,

15 c.c. of distilled water was shaken with o.toluidine. When 3 drops of phenolphthalein were added to the filtrate, the indicator did not turn pink and it required 0.40 c.c. of M/100 NaOH to bring the fluid to the same pink color obtained in I above.

When the same procedure was applied to a M/10 KI solution, the following results were obtained.

- I. 15 c.c. ortho toluidine + 15 c.c. of M/10 KI
were shaken for two days
10 c.c. of filtrate + 3 drops of phenolphthalein
pink color - similar to that of KCNS.
- II. 15 c.c. of ortho toluidine + 15 c.c. of distilled H₂O
were also shaken for two days
10 c.c. of filtrate + 3 drops of phenolphthalein required
the addition of 0.32 c.c. M/100 NaOH in order to
equal the pink color of I above.

From the experiments with organic bases, one can accept the following conclusions:

1. An inverse electromotive force effect is obtained opposite to that of organic acids.
2. A chemical reaction occurs between the organic base and the salt solution at their phase boundaries, whereby a base is liberated.

It is not surprising that such a liberation of a base is not readily observed in some cases, since the electromotive force effect of concentration is not always readily observed due to the presence of impurities. Much difficulty may be encountered in producing this effect due to the presence of impurities. Variation in the types of impurities found in different samples of toluidine influences the results markedly.

EXPERIMENTAL EVIDENCES AGAINST MICHAELIS' PORE THEORY.

Experimental Evidences Against Michaelis' Pore Theory.

The phase boundary potential theory caused some disagreement among biophysicists. L. Michaelis and his coworkers were its chief opponents. In 1925 Michaelis introduced the diffusion potential theory (4-12) as an explanation for the mode of action of membranes. This theory attributed the potential differences produced by membranes to the difference in velocity of the single ions diffusing across the membrane. Furthermore, Michaelis claims that there is no abrupt change in the potential at the boundary of the membrane, but a gradual change of the potential from the one boundary towards the other, as in the case of a liquid junction potential when two electrolytic solutions are in direct contact with each other, and by diffusion or convection a gradual transition between the two solutions is interposed.

To explain this theory of diffusion potential, Michaelis used a dried collodion membrane. This membrane Michaelis said, acted as a molecular sieve, i.e., the retention of ions by an oppositely charged molecular wall. He observed that well dried collodion film showed an electrode-like electromotive action and that these forces came about not through a phase boundary potential but by means of electrically charged pores which act as an ionic filter. However, Michaelis was well aware of the possibility of explaining such an electromotive force as a re-

sult of phase boundary potential, for this system

- conc. NaCl	certain water immiscible fluids like salicylic aldehyde	dil. NaCl +
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had a similar electromotive force. He even admitted that this explanation was not necessarily to be applied to the electrode-like action of plant cuticula. Nevertheless, Michaelis claimed that the theory of phase boundary potential could not be applied to the dried collodion film for the following reason, which will be stated in his own words (5).

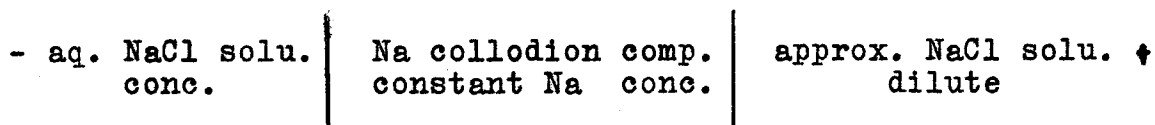
"A completely dried collodion membrane is a perfect electric insulator and is highly electrified by gently rubbing it against the hair. Not being easily wetted by water, it retains its electric charge a very long time even in a humid atmosphere. This property of an electric insulator may be emphasized because it shows that the properties of permeability and electric conductivity are due to the pores and their contents and not to the chemical or physical nature of the solid nitrocellular substance. When such a membrane is thick enough and separates two electrolytic solutions, the conductivity is, at the beginning, almost zero. It increases, however, rapidly with the progress of imbibition by the membrane. Now it would be absurd to suggest that water or a potassium chloride solution could go into "solid solution" in the dried collodion within some minutes."

Thus Michaelis, in his experimental work was able to say that this dried collodion membrane acted as a molecular sieve, retaining ions of one kind due to the charge of the membrane wall, and that it would be ridiculous to suggest that water or a potassium chloride solution can go into solution in this membrane within some minutes.

Michaelis' arguments are not convincing enough since experimental evidences show that not only a "going into solution" occurs but also chemical reactions can be very easily demonstrated. John H. Northrop, in his article on "The Permeability of Thin Dry Collodion Membranes" (13,14) states that gases do not pass through holes in the collodion membrane, for the rate of diffusion would then be inversely proportional to the square root of their density and experiments show that a number of gases pass much more rapidly than hydrogen. Taken as a whole, results indicate that the substance first dissolves in and then diffuses through the membrane. Thus water, also must dissolve in the substance of collodion which would account for the increase of conductivity even if the filling of the pores played no part. This proof favors the assumption of the "going into solution" in the collodion rather than of a passage through the pores.

Collodion or nitrocellulose is a highly reactive substance and it is capable of entering into surface reactions with aqueous salt solutions like KCl, and NaCl whereby HCl and a sodium compound of nitrocellulose are formed. Thus the presence of a chemical reaction at the surface of the dried collodion film can be very easily demonstrated. Furthermore, the theory of phase boundary potential is very applicable in this case. When NaCl is shaken with collodion, in a shaking machine, for several days, the Na^+ ion attaches itself to the collodion and

a constant Na^+ ion concentration is present in the collodion. This produces an electromotive force similar to the one observed by Michaelis because the Na^+ ion concentration is thus kept constant at the surface of the dried collodion film and the following cell system was set up.



Titration experiments were performed by R. Beutner showing the liberation and the detection of HCl and the following conclusions were accepted by him.

1. Collodion will give off traces of acid when shaken with salt solutions.
2. Collodion will even give off slight traces of acid when shaken with water. This is probably due to impurities contained in the water.

So far it has been shown that there is a "going into solution" in the collodion membrane and that there is a chemical reaction occurring on the surface of the collodion film between the collodion and the common ion of the aqueous solution. These evidences weaken L. Michaelis' pore theory of diffusion potential theory immensely.

There are, moreover, a few other arguments in opposition to Michaelis' pore theory.

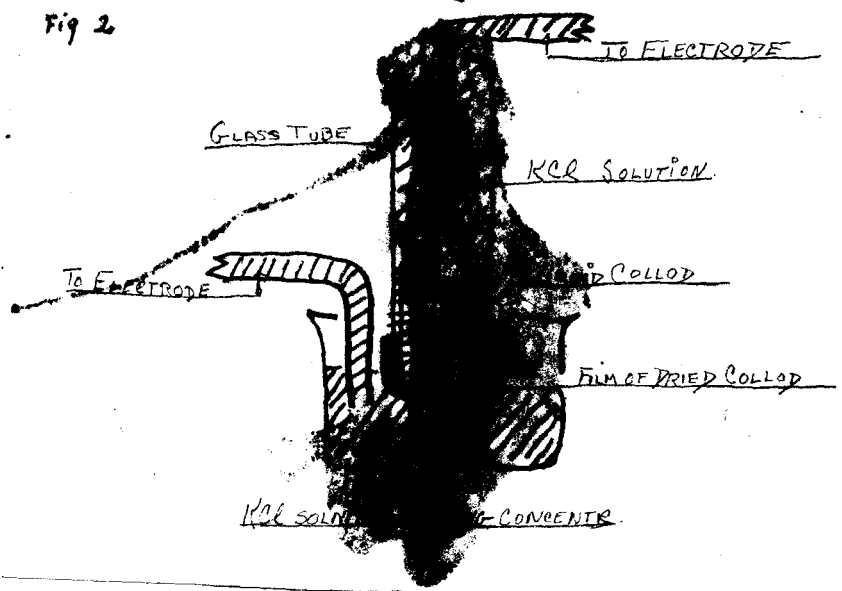
- 1) Michaelis claims that if two KCl solutions of varying concentrations are separated by a dried collodion membrane, a dif-

ference in potential will be produced due to the filling of the pores of the membrane from opposite sides. Suppose, however, we insert liquid collodion, ether and alcohol (see diagram below) between one of the KCl solutions and the collodion membrane, then the filling of the pores with KCl solutions from opposite sides should be obviated and no influence of the concentration upon the electromotive force of this system should appear according to the pore theory. But experimental evidences show that when the KCl concentrations were changed the electromotive force varied in the same manner as on an ordinary dried collodion film, hardly making any difference which solution was first applied.

The following cell system was set up.

KCl + liquid collodion ether and alcohol	solid dried collodion	KCl of varying conc.
---	--------------------------	----------------------

Fig 2



Experimental Results

Membrane plus a 2 mm column of collodion solution was used.

Membrane #1

Elec.	System	Elec.								
Left	Membrane #1	rt.	down	up	down	up	down	up	down	Ave.
+	$\frac{M}{10} KCl / \frac{M}{10} KCl$	—	82	80	83	82	81	82	82	82
+	$\frac{M}{10} KCl / \frac{M}{100} KCl$	—	112	110	113	114	112	113	112	112
+	$\frac{M}{10} KCl / \frac{M}{1000} KCl$	—	155	153	153	157	156	157	154	155
+	$\frac{M}{10} KCl / \frac{Dist.}{H_2O}$	—	148	150	146	146	148	148	147	148

Same method was applied except measurements were made, first, with very dilute solutions and then with highly concentrated ones.

Membrane #2

Left	Membrane #2	rt.	down	up	down	up	down	up	down	Ave.
+	$\frac{M}{10} KCl / \frac{M}{1000} KCl$	—	149	151	153	154	152	151	152	151
+	$\frac{M}{10} KCl / \frac{M}{100} KCl$	—	106	110	110	109	107	108	110	109
+	$\frac{M}{10} KCl / \frac{M}{10} KCl$	—	79	78	81	81	82	80	82	80
+	$\frac{M}{10} KCl / \frac{Dist.}{H_2O}$	—	147	148	149	148	147	147	147	147

From these results one can readily see that the liquid collodion used between the KCl solution and the collodion membrane does not stop changes in the electromotive force when the concentrations of KCl solutions are varied. According to Michaelis'

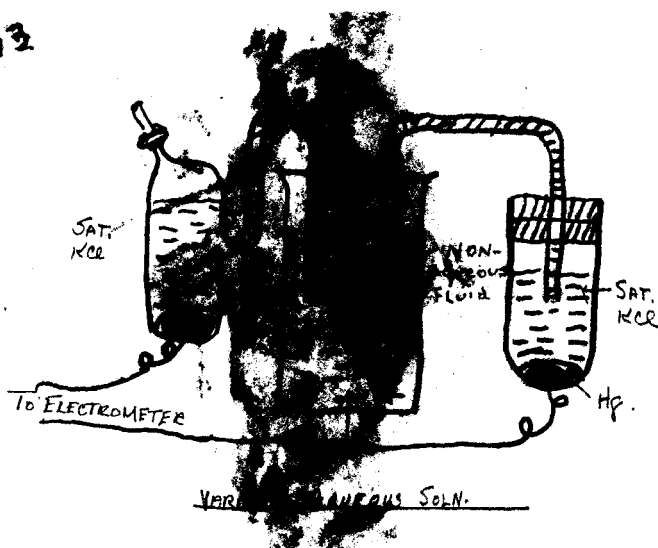
pore theory, no variation in the electromotive force should occur in a cell system set up in this manner.

One may account for this discrepancy by the assumption that there are some pores left and that these are filled on the first contact with a given KCl solution which stays in the pores and comes into contact with the solutions subsequently applied. If this is the explanation, the observed electromotive force ought to depend on the concentration which was first applied. Our experimental results, as already seen above, show that this is by no means the case.

2) Another consideration against Michaelis' pore theory. Michaelis favors the diffusion potential theory against R. Beutner's phase boundary potential theory. If Michaelis' theory were correct, then the electric potential of a mixture of two electrolytes separated by a non-aqueous water immiscible substance should be a middle value of the value of the two components. Experimental evidences, however, show that this is not true.

An apparatus as shown in Fig. 3 was used.

Fig. 3



The hook-shaped electrode was filled with water immiscible oily liquids and immersed first in a M/10 HCl solution, then into a KCl solution and finally into a mixture of equal parts of HCl and KCl solutions. The electromotive force of each substance was measured.

Guaiacol

M/10 HCl	- 0.005	Volts
M/20 HCl + M/20 KCl	- 0.003	"
M/10 KCl	+ 0.020	"

O. Cresol

M/10 HCl	+ 0.064	"
M/20 HCl + M/20 KCl	+ 0.060	"
M/10 KCl	+ 0.079	"

M. Cresol

M/10 HCl	- 0.017	"
M/20 HCl + M/20 KCl	- 0.016	"
M/10 KCl	+ 0.004	"

Celloidin

M/10 HCl	- 0.070	"
M/20 + M/20 KCl	- 0.057	"
M/10 KCl	+ 0.006	"

From these experimental results, one readily observes that the electric potential of a mixture at a true phase boundary is not a middle value of the values of the two components, but is always nearer to the value of the more penetrating salt.

It is interesting to note how potential differences between miscible solutions (usually known as diffusion potentials) produce different results. The electric potential of mixtures is found to be nearer the middle value of the values of the two components. Experimental evidences prove this to be true.

The same apparatus with the hook-shaped electrode is used in these experiments.* The only difference in this system is that the cup attached to the hook-shaped tube instead of being filled with a water immiscible oily substance is now filled with concentrated HCl solution held in place by Gravel.

Gravel electrode Experiment

with dilute HCl in the beaker	E.M.F.	-0.034 volts
" " KCl " " "	"	+0.050 "
" a mixture of KCl & HCl	"	+0.014 "

The electric potential of the mixture of KCl and HCl is a middle value of the two components.

* See page 26.

If Michaelis' theory were correct then a mixture of KCl and HCl using collodion as a membrane, should produce an electric potential close to the middle value of the values of the two components KCl and HCl. Observations show us, however, that this is not the case.

(1) with dilute HCl in contact with a collodion film = - 0.070 v.

+	inside	collodion	outside -
	KCl	membrane	HCl
	(1 mol)		.1 M

(2) with dilute KCl in contact with a collodion film = + 0.006 v.

inside	collodion	outside
KCl	membrane	KCl
.1M		.1 M

(3) with a mixture of KCl and HCl = - 0.057 v.

inside	collodion	outside mixture
KCl	membrane	HCl & KCl
.1 M		M/20 M/20

SUMMARY.

1. Organic acids, like salicylic aldehyde and cresol plus oleic acid, when interposed between aqueous solutions of different concentrations, will produce electromotive forces in a similar order of magnitude to those which appear in living tissues.
2. There is a chemical reaction occurring at the phase boundary of the cresol plus oleic acid and various salt solutions.
3. Organic bases produce inverse electromotive force effects opposite to that of organic acids.
4. A base is liberated when certain salt solutions like KI or KCNS are shaken with ortho toluidine. This is due to the high penetrability of the I⁻ and CNS⁻ ions in the ortho toluidine.
5. Experimental evidences against Michaelis' pore theory are:
 - a) Collodion is not an inert substance; there is a "going into solution" in the collodion membrane and a chemical reaction also occurs at the surface of the collodion film and the various salt solutions.
 - b) Liquid collodion, inserted between the KCl solution and the collodion membrane, does not prevent any changes in the electromotive force when the concentrations of KCl solutions are varied.

- c) The electric potential of a mixture of two electrolytes separated by a non-aqueous water immiscible substance is not a middle value of the value of the two components.

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